Protective Effect of 4-Methoxy Benzyl Alcohol on the Blood–Brain Barrier after Cerebral Ischemia Reperfusion Injury

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Damage of the blood–brain barrier (BBB) during the process of cerebral ischemic injury is a key factor that influences the therapeutic efficacy to the cerebral ischemic injury. This work was designed to investigate the mechanisms underlying the protective effects of 4-methoxy benzyl alcohol (4-MA) on the BBB by developing a cerebral ischemia/reperfusion model of rats (MCAO/R). The MCAO/R was developed through a thread embolism method. The neurologic scales, the brain infarct rate, and the Evans blue (EB) contents of the brains were detected. Meanwhile, the release of nitric oxide (NO) and activities of NO synthase (NOS) in brain tissues were measured. Western blotting analyses were also used to assess the protein expressions of aquaporin-4 (AQP-4), occludin, and claudin-5 in brain tissue. After rats were pretreated with different concentrations of 4-MA, the neurologic scores, the infarct rate, and the EB contents in the brain tissues were significantly decreased. The release of NO and the activities of neuronal NOS and inducible NOS were notably inhibited. Furthermore, the protein expression of AQP-4 was markedly decreased, whereas the protein expressions of claudin-5 and occludin were significantly increased. In conclusion, the 4-MA decreases the permeability of BBB when focal cerebral ischemia occurs. The inhibition of the NOS pathways, the attenuation of the protein expression of AQP-4, and the enhancement of the expressions of the tight junction proteins might contribute to the protective effects of 4-MA on the BBB. **Key Words:** 4-Methoxy benzyl alcohol—blood–brain barrier—nitric oxide synthase—tight junction—aquaporin-4.

Introduction

As a traditional Chinese medicinal herb, *Gastrodia elata* Blume (GEB) was documented to treat many symptoms in the nervous system, such as language paralysis, trance, headache, and dizzy spin, which commonly occur in cerebral ischemic injury (CIRI). Reports also suggested that GEB and its compounds were helpful for clinical neural functional recovery and improved daily life in people who have stroke.[1-3] Meanwhile, the anti-ischemic stroke effect of GEB and its ingredients was experimentally proven.[4,5]

Our previous studies showed that the ethyl acetate extracts of GEB significantly protected the cerebrum from ischemic injury in a cerebral ischemia reperfusion animal model (MCAO/R).[6] Further studies found that ethyl acetate extracts of GEB significantly inhibited platelet aggregation, prevented hippocampal CA1 cell from death, and attenuated the generation of nitric oxide (NO) in the brain.[7-9] All these previous studies suggested that GEB could exert protection on the blood–brain barrier (BBB).[10] 4-Methoxy

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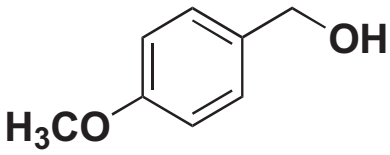

Figure 1. *The structure of 4-methoxy benzyl alcohol (4-MA).*

benzyl alcohol (4-MA, Fig 1) is a major active compound in GEB; 10 kg of dried GEB contains about 1 g of 4-MA (Xiao cao ba, Zhao tong, China) was purchased from J&K Scientific (Beijing, China).[11] Previous evidence suggested that 4-MA was able to cross the BBB easily both in normal rats and in rats in the MCAO/R group, and remained in brain tissue and cerebrospinal fluid for a long time after MCAO/R.[12,13] Further study found that the compound was able to significantly inhibit platelet aggregation, relax vascular smooth muscle, and quench inflammation (Li et al., 2017, unpublished). However, the protective role of 4-MA on the BBB remains to be elucidated (Fig 2).

The BBB is a physical barrier between the blood and the neurons. Disruption of the BBB is a critical event during CIRI followed by passive diffusion of water, leading to vasogenic edema and secondary brain injury.[14-16] After CIRI, the BBB is damaged at structural and functional levels, including endothelial cell pyknosis, capillary basement membrane rupture, and blood capillary lumen narrowing.[14] Eventually, the increase of BBB permeability leads to brain edema and results in nerve damage, which is often considered as secondary brain injury.[17,18] Improvement of cerebral edema is one of the most important treatments for reducing subsequent chronic neural damage in stroke, and suggesting molecular water transport has generated great interest in new strategies for brain edema therapy.[19] Because sham of the pathophysiological process of BBB damage may play a critical role in the treatment and prognosis of CIRI, several studies reported that dysfunction of the BBB after ischemic stroke was attributed to the attenuation of vascular endothelial cell tight junction (TJ), degradation of the major structural protein (occludin and claudin-5), overexpression of aquaporin-4 (AQP-4), and inflammation reaction.[20-23] Therefore, the present study aimed to illustrate that the mechanism underlying the protective effects of 4-MA on the BBB in MCAO/R rats could be associated with the NO pathway.

Materials and Methods

Development of Rat MCAO/R

Adult male Sprague-Dawley rats (weighing 250-300 g) were provided by the Laboratory Animal Center of Experimental Animal Center (Sichuan, China) and housed under diurnal lighting conditions (12-hour light–dark cycle). All experimental protocols and animal handling procedures were performed in accordance with the Experimental Animal Ethics Committee of Yunnan University of Traditional Chinese Medicine.

The MCAO/R model was developed as described previously.[24-26] Briefly, the animals were intraperitoneally anesthetized with 10% chloral hydrate (.3 mL/100 g). The skin was opened, and the left common carotid artery (CCA) and the external carotid artery were exposed. Then, a 3-0 surgical monofilament nylon suture (Prodo Co., Ltd., Tokyo, Japan) was carefully inserted into the vessel from the CCA to the internal carotid artery, and reached the root of the left middle cerebral artery until a light resistance was felt (18-22 mm from CCA bifurcation), then the blood flow was occluded. After 2 hours of MCAO, the nylon suture was removed to restore blood flow (reperfusion) for 24 hours of reperfusion. Rats in the sham group underwent the surgery without suture insertion. The rats were placed in cages for recovery after incision closure, with free access to food and water. Body temperature was maintained at 37.0 ± .5°C using a heating pad and air conditioner during the whole procedure.

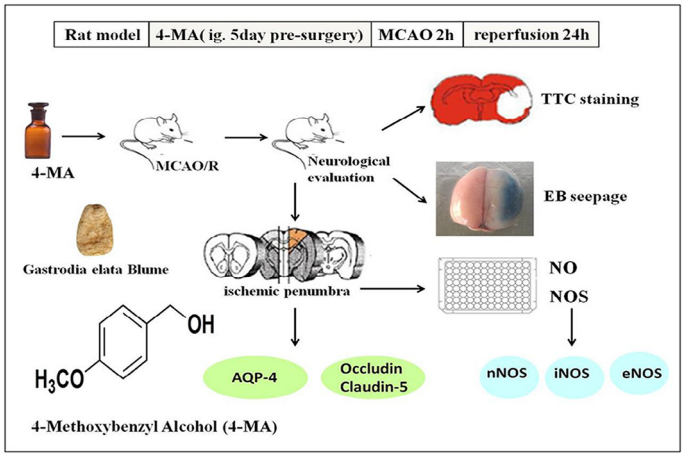

Figure 2. *Experimental protocol.4-MA (10 and 20 mg/kg) was administered via gavage for 5 days (q.d.) 30 min prior to surgery in rat MCAO/R model. Experimental protocol: protective effects of 4-MA on cerebral ischemia and reperfusion injury are associated with blood-brain barrier protective. 4-MA, 4-Methoxy benzyl alcohol; MCAO/R, middle cerebral artery occlusion and reperfusion; TTC, 2,3,5-triphenyltetrazolium chloride; EB, evans blue.*